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CHROMOSOMES OF NORTH AMERICAN GRAPEFERNS AND MOONWORTS (OPHIOGLOSSACEAE: BOTRYCHIUM)

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Because of rarity and small size, and the resulting inconspicuousness, the diversity of moonwort species (*Botrychium* subg. *Botrychium*) has been largely overlooked by botanists, until the last 15 years. Intensive work since 1977 has resulted in discoveries, descriptions, and reinterpretations of 16 species to be added to the seven known before.

Scattered reports of chromosome numbers in a few of the more common moonworts have appeared in the past. Many of these, however, have been published without voucher citations and have lacked illustrations of the chromosomes. Table 1 gives chromosome numbers for all of the described North American botrychiums, with the exception of *Botrychium gallicomontanum* (Farrar & Johnson-Groh 1991). Photographs and, in two cases, interpretive drawings of those taxa not previously illustrated are shown in Figs. 1–4.

A number of the new species whose chromosome numbers are given here are found only in the western mountains of North America, and many grow at high altitudes. The stages of sporogenesis suitable for meiotic counts occur early in the spring, and in the mountain habitats, snow-covered roads make collecting chromosome material prohibitive. In order to solve this problem our method was to collect clumps of soil containing mature moonworts during the summer collecting fieldwork. The soil clumps in plastic bags were kept in a Cold Temperature Room (40° F) during the winter, and in the spring placed in empty covered glass aquaria outdoors. Each clump was watered on alternate days. The botrychiums would emerge, produce young sporangia, which were fixed in Newcomer's Solution, and then be collected as vouchers if they matured. We have found it difficult, if not impossible, to cultivate these plants for more than a year, but we were ultimately successful using this method in obtaining chromosome numbers for all the western species and also for those that occur in the Lake Superior area.

The cytological procedure consisted of placing young sporangia on a microscope slide in a drop of ACH (50% aceto-carmine, 50% Hoyer's Solution), teasing and removing the sporangial walls, and, after covering with a cover slip, gently squashing the remaining contents. Good figures were photographed with a compound microscope under an oil immersion 100× objective, or a 40× high dry objective if the figure was too large. Interpretive drawings were made with a drawing tube to facilitate counting the chromosomes. Slides were sealed first with nail polish and then, after a week, with Glyptal (a sealant made by General Electric). In the case of *B. montanum,* the only figure obtained was photographed but could not be drawn; the number is therefore approximate. The chromosomes of *B. crenulatum*, on the other hand, are shown in a drawing, the figure not surviving to be photographed.

TABLE 1. Chromosome numbers in North American botrychiums. All Wagner vouchers are deposited at MICH.

Species	Locality	Chromosome Number	Ploidal Level	Reference
Botrychium subg. *Osmundopteris*				
B. virginianum (L.) Sw.	MI: Washtenaw Co., *Wagner* on May 15 1954	$n = 92$	4x	Wagner 1955
Botrychium subg. *Sceptridium*				
B. biternatum (Sav.) L. Underw.	IN: Jefferson Co., *Wagner 9273*	$n = 45$	2x	Wagner 1963
B. dissectum Spreng.	*Britton s.n.*	$n = 45$	2x	Britton 1953
B. jenmanii L. Underw.	AL: Mobile Co., *Beibel 9213*	$n = 90$	4x	Wagner 1963
B. multifidum (Gmel.) Rupr.	MI: Washtenaw Co., *Wagner* on 22 June 1954	$n = 45$	2x	Wagner 1955
B. oneidense (Gilb.) House	MI: Washtenaw Co., *Wagner* in 1954.	$n = 45$	2x	Wagner 1955
B. rugulosum W. H. Wagner	MI: Monroe Co., *Wagner 9067*	$n = 45$	2x	Wagner & Wagner 1982
B. lunarioides (Michx.) Sw.	AL: Lee Co., *Wagner 9195*	$n = 45$	2x	Wagner 1963
Botrychium subg. *Botrychium*				
B. acuminatum W. H. Wagner	MI: Alger Co., *Wagner 85043*	$n = 90$	4x	Wagner & Wagner 1990b
B. ascendens W. H. Wagner	OR: Wallowa Co., *Wagner 83363*	$n = 90$	4x	Wagner & Wagner 1986
B. campestre W. H. Wagner & Farrar	MI: Leelanau Co., *Wagner 85025*	$n = 45$	2x	Wagner & Wagner 1986
B. crenulatum W. H. Wagner	CA: San Bernardino Co., *Kiefer* on 15 Aug 1963	$n = 45$	2x	Wagner & Wagner 1981
B. echo W. H. Wagner	CO: Clear Creek Co., *Wagner 82135*	$n = 90$	4x	Wagner & Wagner 1983
B. gallicomontanum Farrar & Johnson-Groh	MN: Norman Co.	unknown	4x	Farrar & Johnson-Groh 1991

TABLE 1 continued.

B. hesperium (Maxon & Clausen) W. H. Wagner & Lellinger	CO: Clear Creek Co., *Wagner 82136*	$n =$ 90	4x	Wagner & Wagner 1983
B. lanceolatum (Gmel.) Angstr. ssp. *lanceolatum*	WA: Skamania Co., *Wagner 63112*	$n =$ 45	2x	Wagner in Fabbri 1963
B. lanceolatum ssp. *angustisegmentum* (Pease & Moore) Clausen	MI: Emmet Co., *E. G. Voss* on 15 May 1954	$n =$ 45	2x	Wagner 1955
B. lunaria (L.) Sw.	MI: Chippewa Co., *Wagner 8199*	$n =$ 45	2x	Wagner & Lord 1956
B. matricariifolium A. Br.	MI: Emmet Co., *Wagner* in 1955	$n =$ 90	4x	Wagner & Lord 1956
B. minganense Victorin	MI: Chippewa Co., *Wagner 8200*	$n =$ 90	4x	Wagner & Lord 1956
B. montanum W. H. Wagner	MT: Lake Co., *Wagner 80110*	$2n =$ ca. 90	2x	Wagner & Wagner 1981
B. mormo W. H. Wagner	MN: Clearwater Co., *Wagner 77326*	$2n =$ 90	2x	Wagner & Wagner 1981
B. pallidum W. H. Wagner	MI: Chippewa Co., *Wagner 89041*	$2n =$ 90	2x	Wagner & Wagner 1990a
B. paradoxum W. H. Wagner	AB: Waterton Lakes Natl. Park , *Wagner 83331*	$n =$ 90	4x	Wagner & Wagner 1981
B. pedunculosum W. H. Wagner	OR: Wallowa Co., *Wagner 83361*	$n =$ 90	4x	Wagner & Wagner 1986
B. pinnatum St. John	WA: Skamania Co., *Wagner 63111*	$n =$ 90	4x	Wagner in Fabbri 1963
B. pseudopinnatum W. H. Wagner	ON: Thunder Bay Dist., *Drife* in 1989	$n =$ 135	6x	Wagner & Wagner 1990b
B. pumicola Coville	OR: Klamath Co., *R. M. Brown* in 1954	$n =$ 45	2x	Wagner 1955
B. simplex E. Hitchc.	MI:Washtenaw Co., *Wagner 8142*	$n =$ 45	2x	Wagner 1955
B. spathulatum W. H. Wagner	ON: Thunder Bay Dist., *Wagner 88036*	$n =$ 90	4x	Wagner & Wagner 1990a
B. ×*watertonense* W. H. Wagner (*hesperium* × *paradoxum*)	AB: Waterton Lakes Natl. Park, *Wagner 83332*	$2n =$ 180 (meiosis irreg.)	4x	Wagner et al. 1984

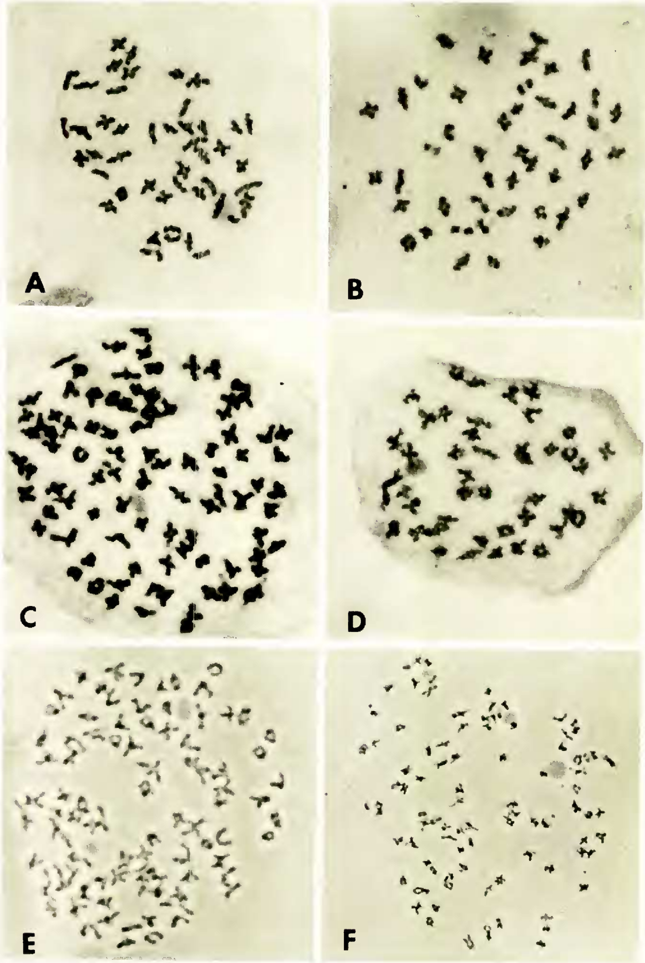

FIG. 1. Meiotic chromosomes of *Botrychium*; localities and voucher numbers are shown in Table 1. A. *Botrychium biternatum*, $n = 45$. B. *B. rugulosum*, $n = 45$. C. *B. jenmanii*, $n = 90$. D. *B. lunarioides*, $n = 45$. E. *B. echo*, $n = 90$. F. *B. ascendens*, $n = 90$.

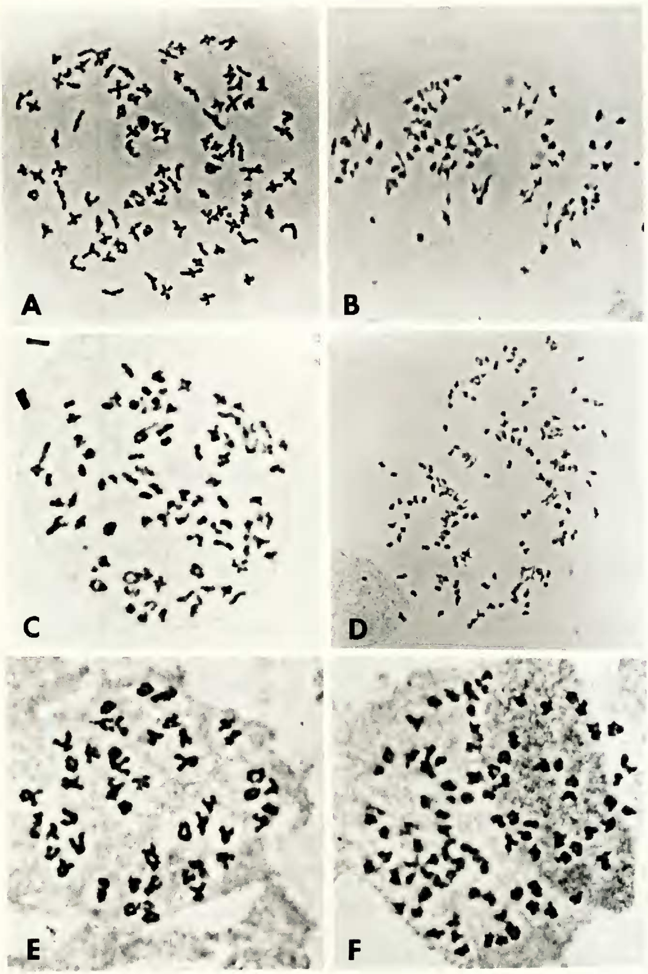

FIG. 2. Meiotic chromosomes of *Botrychium*; localities and voucher numbers are shown in Table 1. A. *Botrychium hesperium*, *n* = 90. B. *B. paradoxum*, *n* = 90. C. *B. pedunculosum*, *n* = 90. D. *B.* ×*watertonense*, ca. 41 II's, 99 I's. (Explanatory drawing in Fig. 4B). E. *B. lanceolatum* subsp. *lanceolatum*, *n* = 45. F. *B. pinnatum*, *n* = 90.

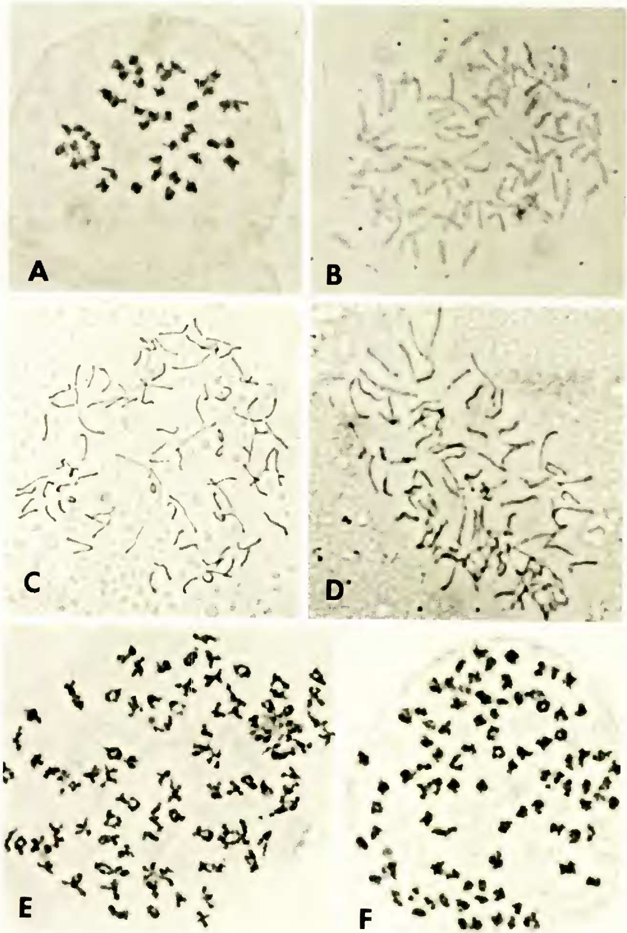

FIG. 3. Meiotic and somatic chromosomes of *Botrychium*; localities and voucher numbers are shown in Table 1. A. *Botrychium campestre*, $n = 45$. B. *B. mormo*, $2n = 90$. C. *B. pallidum*, $2n = 90$. D. *B. montanum*, $2n =$ ca. 90. E. *B. spathulatum*, $n = 90$. F. *B. acuminatum*, $n = 90$.

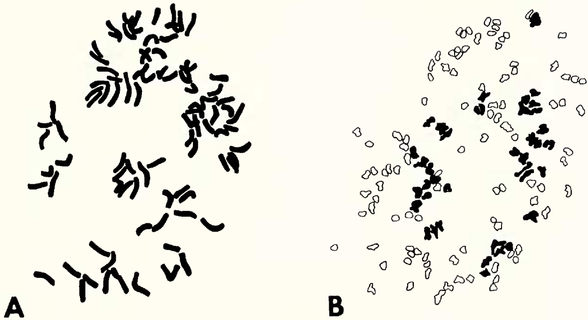

FIG. 4. A. Chromosomes of *Botrychium crenulatum*, $2n = 90$; locality and voucher number are shown in Table 1. B. Explanatory drawing of Fig. 2D, chromosomes of *B.* ×*watertonense*, ca. 44 II's and 99 I's.

The three subgenera of *Botrychium* in North America are *Osmundopteris*, *Sceptridium*, and *Botrychium*. (A fourth subgenus, *Japanobotrychium*, occurs in the Old World tropics.)

Subgenus *Osmundopteris*: *Botrychium virginianum* (L.) Sw., the most familiar and one of the most widespread species in the genus, occurs in Asia, Europe, North America, and extends as a morphological cline into South America. The chromosome number is tetraploid, with $n = 92$ ($x = 46$), an unusual base number, the only one in a genus in which almost all of the numbers are based on $x = 45$. The other member of subg. *Osmundopteris*, *B. strictum* L. Underw., from eastern Asia, also has an aberrant number, $n = 44$ (Sahashi 1982).

Subgenus *Sceptridium*: The evergreen grapeferns number approximately 25 species, seven of which occur in North America. Six of these are diploid with a chromosome number of $n = 45$. The seventh, *Botrychium jenmanii* L. Underw. (syn. *B. alabamense* Maxon), is a tetraploid with $n = 90$, a presumed ancient allopolyploid hybrid of the diploid species, *B. biternatum* (Sav.) L. Underw. and *B. lunarioides* (Michx.) Sw. (Wagner 1963). It occurs also in the Caribbean and Central America. No other potential hybrids are known in the North American members of the subgenus. There is, however, an undescribed taxon of *Sceptridium* in Kentucky and Tennessee, whose chromosome number and ploidal level are as yet undetermined.

Subgenus *Botrychium*: All 23 taxa of the moonworts have chromosome numbers based on 45. Surprisingly, half of the members of the subgenus are tetraploids, and one is a hexaploid. Until fairly recently only five taxa of moonworts were known in eastern North America, *B. simplex* E. Hitchc., *B. lunaria* (L.) Sw., *B. lanceolatum* (Gmel.) Angstr. (subsp. *angustisegmentum* (Pease & Moore) Clausen), *B. matricariifolium* A. Br., and *B. minganense* Victorin, and three in the West, *B. lanceolatum* (subsp. *lanceolatum*), *B. pumicola* Coville, and *B. pinnatum* St. John (formerly confused with *B. boreale* Milde of the Old World). Many of the 16 new species were simply overlooked or thought to be forms of the more common

TABLE 2. Sterile hybrid botrychiums in North America.

Species	Locality	Reference
B. ascendens × *crenulatum*	OR: Wallowa Co. *Wagner 83363a*	Wagner & Wagner 1986
B. echo × *minganense*	AZ: Apache Co. *Wagner 82104*	Wagner & Wagner 1983
B. lanceolatum × *minganense*	CO: Gunnison Co. *Wagner 82121*	Wagner & Wagner 1988
B. lanceolatum ssp. *angustisegmentum* × *matricariifolium*	MI: Alger Co. *D. Henson 2300*	Wagner & Wagner 1988
B. lunaria × *pinnatum*	BC: Bryan Boru Ck. *J. W. Easton* (UBC)	(Unreported)
B. lunaria × *simplex*	MI: Delta Co. *R. B. Wilson* in 1964	Wagner & Wagner 1988
B. lunaria × *spathulatum*	ON: Thunder Bay Dist. *Wagner 87237*	Wagner & Wagner 1988
B. matricariifolium × *minganense*	MI: Schoolcraft Co. *Wagner 81045*	Wagner & Wagner 1988
B. matricariifolium × *simplex*	MI: Midland Co. *Wagner 8997*	Wagner 1980, 1991
B. pedunculosum × *pinnatum*	OR: Wallowa Co. *Wagner 83361*	Wagner & Wagner 1986
B. ×*watertonense* W. H. Wagner (*hesperium* × *paradoxum*)	AB: Waterton Lakes Natl. Park *Wagner 83332*	Wagner et al. 1984

species, usually *B. lunaria* or *B. maticariifolium*. One of the helpful elements in recognizing the distinction of the new species has been the ploidal level, emphasizing the differences, for example, between *B. lunaria*, 2*x*, and *B. minganense*, 4*x*, and between *B. matricariifolium*, 4*x*, and *B. pseudopinnatum* W. H. Wagner, 6*x*.

Although for many years, authors believed that interspecific hybridization does not occur in these plants, we have much evidence now that it does (Wagner et al. 1985). Indeed, we have now encountered ten different widely scattered and sporadic types that appear to be intermediate between well-known species and that possess abortive spores. These are listed in Table 2. We have very little evidence of their chromosome behavior; however, the misshapen and highly variable spores, often very large and spherical among many small and shriveled ones, present strong evidence that meiosis is irregular.

Hybrids in *Botrychium* are rare, however; usually only a single plant is found among hundreds. These have more often been recognized only after large collections have been made and sorted, when they stand out as morphologically intermediate and difficult to assign to known species. So far we have identified only a

TABLE 3. Hypothetical parents for polyploid botrychiums.

Hypothetical parents (All orthospecies except *B. pinnatum*)	Presumed nothospecies
B. biternatum × *lunarioides*	*B. jenmanii*
B. campestre × *lanceolatum*	*B. echo*
B. campestre × *lunaria*	*B. spathulatum*
B. campestre × *simplex*	*B. gallicomontanum*
B. crenulatum × *montanum*	*B. ascendens*
B. lanceolatum × *lunaria*	*B. pinnatum*
B. lanceolatum × *montanum*	*B. pedunculosum*
B. lanceolatum × *pallidum*	*B. matricariifolium* —> *B. acuminatum* (metaspecies)
B. lanceolatum × *simplex*	*B. hesperium*
B. lunaria × *pallidum*	*B. minganense*
B. pinnatum × *simplex*	*B. pseudopinnatum*

few hybrids in the field and, with one exception, we have not succeeded in obtaining meiotic material of hybrids for chromosome study. In order to do so the putative hybrids must be identified as mature plants in the field, well past meiotic stages of sporogenesis, and tagged in the hope they will reappear the following spring, or the clump collected and overwintered as described above. The one exception is *Botrychium* ×*watertonense* W. H. Wagner, the hybrid between *B. hesperium* (Maxon & Clausen) W. H. Wagner & Lellinger and *B. paradoxum* W. H. Wagner, discussed in detail in Wagner et al. 1984. Meiotic counts of *B.* ×*watertonense* were obtained by the latter method. Meiosis proved to be irregular with the number of pairs of chromosomes ranging from 36 to 41 and the number of unpaired chromosomes from 99 to 110, the totals being approximately 180 chromosomes.

The major question involving the polyploids in *Botrychium* is whether they are autopolyploids from single pre-existing divergent species (orthospecies), or allopolyploids of interspecific hybrids (nothospecies). The first author to suggest that a species of moonwort was actually a polyploid hybrid was Meyer (1981). He proposed that *B. matricariifolium* originated as the sterile hybrid of *B. lanceolatum* and *B. lunaria* (1981, pp. 613 ff., figs. 2, 3). His hypothesis, however, is unlikely, because a number of morphological characters do not conform to this interpretation.

Using such characters as (1) trophophore blade cutting and (2) shape; (3) relative development of the basal pinna pair; (4) marginal denticulation; (5) venation pattern; (6) epicuticular wax; (7) relative length of sporophore; and (8) pinnation of sporophore, origins of some of the polyploid taxa can be hypothesized. For some of the polyploids there are no obvious candidates as possible parents. One of these is *B. acuminatum* W. H. Wagner, a rare species of the Lake Superior region. It is very close morphologically in most characters to *B. matricariifolium*, with which it co-occurs. *Botrychium acuminatum* may represent an example of metaspeciation in which divergent evolution has taken place from an allopolyploid nothospecies, in this case, *B. matricariifolium*.

The most remarkable polyploid is *B. paradoxum* W. H. Wagner, which differs from all other known species, diploid or polyploid, and for which there are no known relatives or postulated parental combinations.

Hypotheses of reticulate origin for the various polyploid taxa are listed in Table 3. They are presented here in the hope that they will be tested in various ways, including observations on chromosome pairing behavior in natural backcrosses, comparisons of isozyme patterns of the postulated parents and their allopolyploid presumed descendants, and observations on chloroplast DNA. Also theoretically at least, it should be possible to reproduce the proposed hybridizations experimentally.

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